ANTIOXIDANTS & REDOX SIGNALING
Volume 16, Number 9, 2012

DOI: 10.1089/ars.2011.4074

FORUM REVIEW ARTICLE

Mitochondria and Programmed Cell Death in Parkinson's Disease: Apoptosis and Beyond

Celine Perier,[1,*] Jordi Bové,[1,*] and Miquel Vila[1–3]

Abstract

Significance: Activation of mitochondrion-dependent programmed cell death (PCD) pathways is instrumental to the demise of substantia nigra pars compacta dopaminergic neurons in experimental mouse models of Parkinson's disease (PD). Supporting the relevance of these findings for PD, key molecular elements of this pathogenic cascade have also been demonstrated in postmortem brain samples of PD patients. ***Recent Advances and Critical Issues:*** Mounting evidence indicates that different morphological types of cell death co-exist in the brain of PD patients, all of which may result from the activation of common upstream PCD pathways. Indeed, contrary to initial views, it is now established that the deleterious effects of PCD pathways are not limited to mitochondrion-mediated caspase-dependent apoptosis but also involve caspase-independent nonapoptotic cell death, including necrosis. This notion may help reconcile the observation of both apoptotic and nonapoptotic dopaminergic cell death in postmortem PD samples. ***Future Directions:*** Potential neuroprotective strategies for PD should be aimed at targeting both apoptotic and nonapoptotic pathways, all of which may simultaneously occur in PD patients through activation of common upstream PCD pathways involving the mitochondria. *Antioxid. Redox Signal*. 16, 883–895.

Introduction

Programmed cell death (PCD) is a physiologic process in which molecular programs intrinsic to the cell are activated to cause its own destruction. This process is a fundamental property of all pluricellular organisms and is crucial for their development, organ morphogenesis, tissue homeostasis, and defense against infected or damaged cells. However, excessive PCD or abnormal re-activation of PCD in adulthood can cause unwarranted cell death, which might lead to diseases such as immunodeficiency and neurodegeneration (96).

The term PCD has often been used interchangeably with the term "apoptosis," a particular morphological form of PCD characterized by membrane blebbing, shrinkage of the cell body, nuclear condensation, and DNA fragmentation. However, molecular pathways linked to PCD are implicated in cell death processes, the morphological diversity of which extends beyond apoptosis. Even necrotic cell death, which has been traditionally regarded as an unregulated, passive cell death process lacking morphological features of apoptosis, is now known to occur in part in a regulated (programmed) manner (94). Therefore, the usefulness of the morphological categorization of different types of cell death is being currently debated, and it is expected that biochemical definitions will gradually replace the current vocabulary (47, 94). This notion may also help reconcile the apparently contradictory reports in the literature indicating the occurrence of either apoptotic or nonapoptotic dopaminergic cell death in patients with Parkinson's disease (PD), a debate that has driven the field for several years (96). Based on our current knowledge on the mechanisms of PCD, it is likely that different morphological types of cell death actually co-exist in PD patients, all of which may result from the activation of common upstream PCD pathways.

Molecular Pathways of Apoptosis

Apoptosis can result from the activation of two distinct molecular cascades, known as the extrinsic (or death receptor) and the intrinsic (or mitochondrial) pathways (Fig. 1). The extrinsic pathway is recruited upon activation of cell-surface death receptors, such as Fas/CD95 and the tumor necrosis factor receptor 1 (TNFR1), whereas the intrinsic pathway is triggered by intracellular stimuli such as Ca^{2+} overload or increased generation of reactive oxygen species (ROS). In both pathways, initiator caspases (caspase-8 and −9, respectively) are activated and catalyze the proteolytic maturation of executioner caspases, such as caspase-3, which are the final effectors of cell death.

[1]Vall d'Hebron Research Institute-CIBERNED, Barcelona, Spain.
[2]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.
[3]Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona, Barcelona, Spain.
*These authors contributed equally to this work.

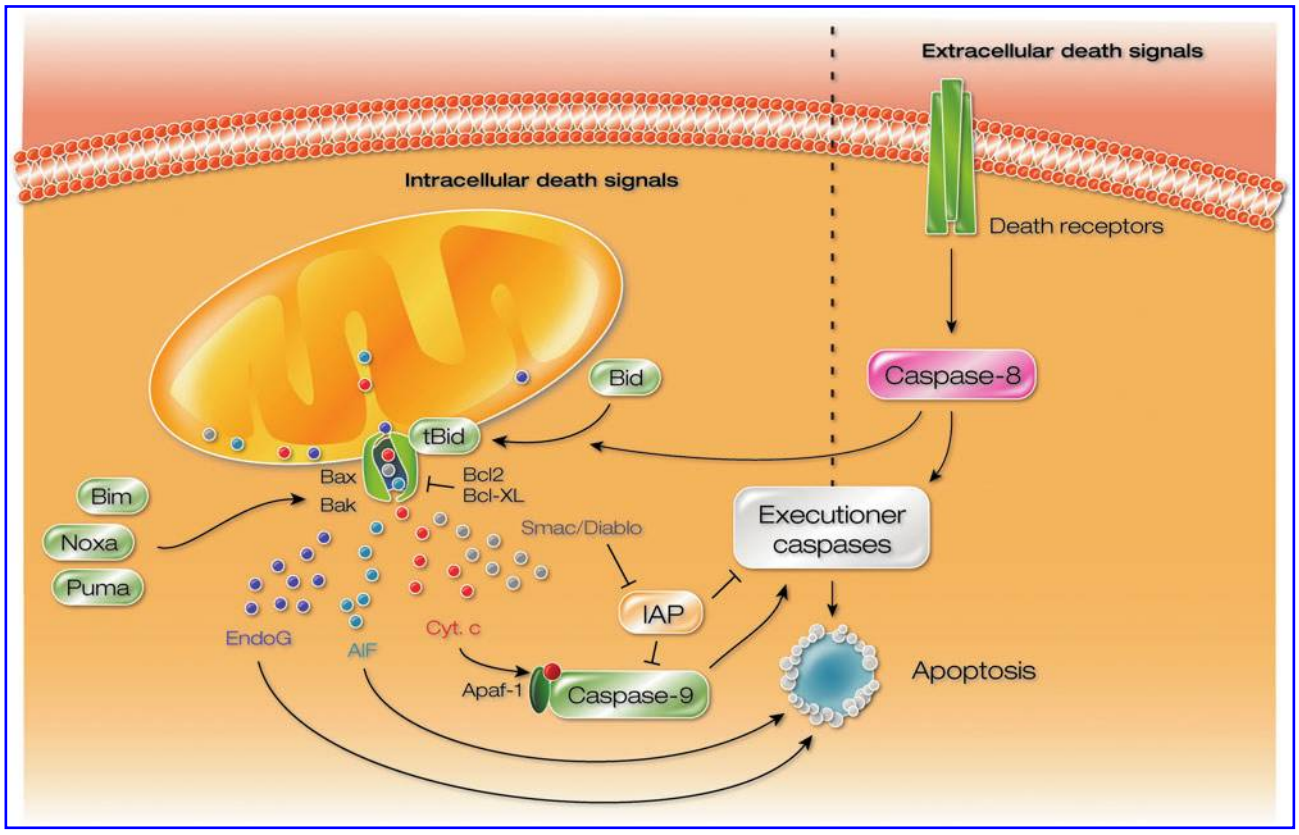

FIG. 1. The extrinsic and intrinsic (mitochondrial) molecular pathways of apoptosis. Extracellular signals through cellular death receptors, such as Fas, and intracellular signals, including damage to subcellular constituents, both trigger genetically programmed pathways of PCD, which converge at the level of mitochondria, leading to caspase-dependent or caspase-independent apoptosis. See main text for details. AIF, apoptosis-inducing factor; Cyt. c, cytochrome; EndoG, endonuclease G; IAP, inhibitor of apoptosis; PCD, programmed cell death; tBid, truncated Bid.

Mitochondrial outer membrane permeabilization (MOMP) represents the point-of-no-return in the mitochondrial apoptotic pathway. Following MOMP, mitochondrial apoptogenic factors such as cytochrome *c*, second mitochondrion-derived activator of caspases (Smac)/direct-IAP binding protein with low PI (Diablo), Omi/high temperature requirement A2 (HTRA2), endonuclease G, or apoptosis-inducing factor (AIF) are released to the cytosol. Once into the cytosol, these factors may initiate cell death in a caspase-dependent or a caspase-independent manner (15). For instance, released cytochrome *c* interacts with two other cytosolic protein factors, Apaf-1 and procaspase-9, to activate caspase-3 (51). On the other hand, Smac/Diablo can interact with several inhibitors of apoptosis (IAPs), thereby relieving the inhibitory effect of IAPs on initiator (*e.g.*, caspase-9) and effector (*e.g.*, caspase-3) caspases (9). In contrast to cytochrome *c* and Smac/Diablo, released AIF and endonuclease G can translocate to the nucleus and induce caspase-independent DNA fragmentation (87). Of note, while all of these mitochondrial intermembrane space proteins were initially identified as apoptogenic, gene-targeted studies in mutant mice indicate that some of them (such as endonuclease G or HtrA2/Omi) appear to be dispensable for cell death (2, 41, 42).

MOMP is highly regulated by proteins of the Bcl-2 family, which comprises several members that either prevent (*e.g.*, Bcl-2 and Bcl-xL) or promote (*e.g.*, Bax and Bak) MOMP and apoptosis (32). Structurally, all these proteins share up to four Bcl-2-homology domains (BH1–BH4). In addition to Bcl-2 family members that contain multiple BH domains, such as Bcl-2 and Bax, there are molecules that share sequence homology only with the BH3 domain (so-called BH3-only proteins). These BH3-only proteins induce cell death either by activating multidomain pro-apoptotic proteins, such as Bax, or by inactivating anti-apoptotic proteins, such as Bcl-2 (50, 104). At least 10 different BH3-only molecules have been described so far in mammals, including BH3-interacting domain (Bid), p53-upregulated modulator of apoptosis (Puma), Noxa (the Greek word for "damage"), or Bcl-2-interacting mediator of cell death (Bim). Bid is activated after its cleavage by caspase-8, thus linking the extrinsic and intrinsic pathways at the level of the mitochondria. Whereas several components of the mitochondrial apoptotic pathway have been implicated in the pathogenesis of PD, the participation of the extrinsic pathway in PD has not been consistently demonstrated (Tables 1 and 2).

Apoptosis and PD

Over the past 10 years, numerous lines of evidence have been gathered indicating that activation of PCD pathways may contribute to substantia nigra pars compacta (SNpc)

Table 1. Targeting Bcl-2-Family Members in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Mouse Model of Parkinson's Disease

Molecule	*Description*	*Type of targeting*	*Effect on MPTP-induced nigro-striatal dopaminergic neurodegeneration*	*Refs.*
Bax	Pro-apoptotic full-length Bcl-2 family member	*Bax* KO	Neuroprotection (cell bodies and terminals)	(95)
Bak	Pro-apoptotic full-length Bcl-2 family member	*Bak* KO	No effect	(25) and UP
Bcl-2	Anti-apoptotic full-length Bcl-2 family member	*Bcl-2* Tg	Neuroprotection (terminals, cell bodies not assessed)	(65, 111)
Bcl-X_L	Anti-apoptotic full-length Bcl-2 family member	Delivery of cell -permeable Bcl-X_L	Neuroprotection (cell bodies, not in terminals)	(22)
Noxa	BH3-only protein	*Noxa* KO	No effect	UP
Puma	BH3-only protein	*Puma* KO	No effect	UP
Bid	BH3-only protein	*Bid* KO	No effect	UP
Bim	BH3-only protein	*Bim* KO	Neuroprotection (cell bodies, not in terminals)	(70)

UP, unpublished personal observation; KO, knock-out; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Tg, transgenic.

dopaminergic neurodegeneration in PD. Initially, the observation of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive dopaminergic neurons in postmortem brains of PD patients was used to support the occurrence of apoptosis in this disease (59). Subsequent studies, using a greater variety of morphological tools, have either succeeded or failed to find more apoptotic neurons in postmortem tissue from PD patients [reviewed in ref. (96)]. This apparent discrepancy can be attributed to both conceptual and technical issues (96). First, it is difficult to detect apoptosis in a particular given time (*i.e.*, death of the patient) due to the presumed low daily rate of neuron cell loss in PD and the presumed rapid disappearance of apoptotic cells. Second, postmortem samples typically derive from advanced stages of the disease, when most of the neurons that are affected by the pathological process are already lost. Third, the TUNEL technique, used in most morphological postmortem studies to determine the presence of apoptotic cells, is not specific for apoptosis, especially in human post mortem tissue, in which factors such as hypoxia can produce TUNEL-positive, nonapoptotic DNA damage (44). Lastly, we now know that common PCD pathways can lead to multiple morphological forms of cell death other than apoptosis, including necrosis, some of which could overlap and co-exist in PD patients (19, 94).

Given these issues, biochemical assessment of molecular components of the PCD machinery has been gradually replacing exclusively morphological approaches. Supporting the implication of PCD pathways in PD, a greater percentage of SNpc dopaminergic neurons positive for pro-apoptotic protein Bax has been observed in brains of PD patients, compared to control subjects (35, 89). Also, increased activity of executioner caspase-3 has been found in SNpc dopaminergic neurons of PD patients (34, 89). Other PCD-related alterations detected in PD brains include the activation of caspase-8 (36, 100) and caspase-9 (100). Despite this body of descriptive data, the actual demonstration that PCD pathways play a pathogenic role in PD-related dopaminergic neurodegeneration has come from experimental animal models, in which elements of the PCD machinery can be manipulated.

Mitochondrial-Mediated Apoptosis in Experimental PD

At present, there are no experimental models that recapitulate all of the clinical and neuropathological features of PD. Instead, there are several experimental systems that allow the exploration of particular aspects of the disease. For instance, genetically modified animals have proven useful to study the function and biology of specific genes linked to familial forms of PD, but less adequate to explore the mechanisms of dopaminergic cell death, as most of these mutant animals do not exhibit consistent neurodegeration. In contrast, mitochondrial parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) reproduces in mice several PD-linked cellular alterations, such as inhibition of mitochondrial complex I (71), increased production of ROS (71, 106), oxidative damage to lipids (71), DNA (38), and proteins (106), including α-synuclein (76), increased levels/cytosolic accumulation of α-synuclein (99), and dopaminergic cell death (18, 96, 98, 99). Most of the *in vivo* mechanistic studies about the involvement of PCD on PD-related dopaminergic neurodegeneration have been performed in MPTP-treated mice, which allows the combination of MPTP intoxication with the use of gene-targeted mutant mice for various PCD molecules.

Using the MPTP mouse model, it has been shown that SNpc dopaminergic neurodegeneration linked to complex I deficiency occurs, at least in part, through activation of mitochondrion-dependent apoptotic molecular pathways (70, 71, 95, 96). Following complex I blockade with MPTP in mice, there is a time-dependent and region-specific mitochondrial release of cytochrome *c* that occurs in association with activation of caspase-9 and caspase-3 (71). Further supporting the induction of apoptosis following complex I inhibition, mitochondrial parkinsonian neurotoxin rotenone induces cytochrome *c* release, caspase-3 activation, and apoptotic cell death in SH-SY5Y cells (67) and ventral mesencephalic dopaminergic neurons (1). In MPTP-treated mice, cytochrome *c* release, caspase activation, and apoptotic cell death were shown to be regulated by pro-apoptotic protein Bax, as they coincided with Bax upregulation and translocation to the mitochondria and were prevented by genetic ablation of Bax (71, 95) (Table 1). The involvement of the mitochondrial-dependent

Table 2. Targeting Other Programmed Cell Death-Linked Molecules in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Mouse Model of Parkinson's Disease

Molecule	*Description*	*Type of targeting*	*Effect on MPTP-induced nigro-striatal dopaminergic neurodegeneration*	*Refs.*
Fas	Initiator of extrinsic pathway	*Fas* KO	Neuroprotection (cell bodies, not in terminals)	(37)
			Increased susceptibility to MPTP (cell bodies and terminals)	(48)
		Immunodeficient *Rag1* KO mice reconstituted with FasL-deficient splenocytes	Neuroprotection (cell bodies, not in terminals)	(7)
TNF	Initiator of extrinsic pathway	*TNFR1* KO, *TNFR2* KO, *TNFR1/2* DKO	No effect	(49, 80)
			No effect (R1, R2 KO), Neuroprotection in DKO (terminals, cell bodies not assessed)	(86)
		TNF-α KO/pharmacological inhibition of TNF-α synthesis	Neuroprotection (terminals, not in cell bodies)	(26)
p53	Proapoptotic transcription factor	*P53* KO	Neuroprotection (cell bodies, not in terminals)	(70, 92)
		Pharmacological inhibition (PFT-α, Z-1-117)	Neuroprotection (cell bodies and terminals)	(23)
JNK	Initiator of apoptosis	*JNK* KO	Neuroprotection (cell bodies and terminals)	(39)
		Pharmacological inhibition (CEP-11004)	Neuroprotection (cell bodies, not in terminals)	(90)
		Pharmacological inhibition (CEP-1347)	Neuroprotection (cell bodies and terminals)	(81)
		Inhibition by adenoviral gene transfer of JNK-binding domain	Neuroprotection (cell bodies and terminals)	(108)
AIF	Caspase-independent executioner of mitochondrion-mediated apoptosis	Harlequin mice with a ~80%–90% reduction in *AIF*	Increased susceptibility to MPTP (cell bodies and terminals)	(69)
Apaf-1	Mediator of cytochrome *c*-induced apoptosis	Inhibition with AAV-mediated delivery of an Apaf-1-dominant negative	Neuroprotection (cell bodies, terminals not assessed)	(60)
Cyclophilin D	Component of mitochondrial PTPC	*Cyclophilin* D KO	No effect	(70)
Caspases	Executioners of apoptosis	Pan-caspase pharmacological inhibition (Q-VD-OPH)	No effect in subacute MPTP; Short-term neuroprotection in acute MPTP (terminals, cell bodies not quantified)	(110)
		Tg of pan-caspase inhibitor *protein baculoviral p35*	Short-term neuroprotection (cell bodies and terminals)	(100)
		Adenoviral gene transfer of *XIAP*	Short-term neuroprotection (cell bodies, not in terminals)	(24)
		Pan-caspase (zVAD-fmk) or caspase-8 (zIETD-fmk) pharmacological inhibition	Switch from apoptotic to necrotic cell death in MPP^+-treated primary ventral midbrain cultures	(36)

AAV, adeno-associated virus vector; AIF, apoptosis-inducing factor; DKO, double knock-out; JNK, c-Jun N-terminal kinase; MPP^+, 1-methyl-4-phenylpyridinium; PTPC, permeability transition pore complex; TNFR1, tumor necrosis factor receptor 1; XIAP, X-chromosome linked inhibitor of apoptosis.

apoptotic pathway in PD-related neurodegeneration is further supported by the neuroprotective effect obtained in MPTP-treated mice by targeting other molecules of this pathway, such as caspase-9 or Apaf-1 (60, 100) or by overexpressing Bcl-2 (65, 111) (Tables 1 and 2). While caspase-8, an initiator caspase of the extrinsic pathway, is also activated in MPTP-treated mice, its activation appears to occur downstream the recruitment of the intrinsic pathway, indicating that this caspase may participate in the amplification, rather than the initiation, of dopaminergic cell death (36, 100). In addition, the BH3-only protein Bid, which cleavage by caspase-8 links the extrinsic pathway with Bax-induced MOMP, appears dispensable for MPTP-induced dopaminergic neuronal death (Table 1).

While complex I inhibition with MPTP leads to mitochondrial-dependent apoptosis, complex I blockade *per se* is not the actual executioner of the cell but rather sensitizes neurons to Bax-dependent cell death through oxidative damage (71). In particular, complex I inhibition by 1-methyl-4-phenylpyridinium ([MPP^+], MPTP's active compound), rotenone, or pathogenic complex I mutations does not directly trigger mitochondrial cytochrome *c* release, but increases the releasable soluble pool of cytochrome *c* in the mitochondrial intermembrane space that can subsequently be released to the cytosol by activated Bax (71) (Fig. 2). This two-step phenomenon is mediated by mitochondrial oxidative damage secondary to complex I inhibition, such as peroxidation of the inner mitochondrial lipid cardiolipin, which disrupts the binding of cytochrome *c* to the mitochondrial inner membrane, leading to an increased soluble pool of cytochrome *c* in the mitochondrial intermembrane space (71) (Fig. 2). In addition to its detachment from the mitochondrial inner membrane, cytochrome *c* release has been shown to require, in other cellular settings, a remodeling of mitochondrial cristae mediated by dynamin-related protein optic atrophy 1 (27, 82).

Activation of Bax after complex I inhibition relies on its transcriptional induction and, most importantly, on its translocation and insertion into mitochondrial membranes, from where it can elicit the release of cytochrome *c* (70, 71, 95). Bax upregulation and post-translational activation in experimental PD involves the cooperation of the transcriptional factor p53 with the BH3-only protein Bim (70). The tumor suppressor protein p53, which is one of the few molecules known to regulate Bax expression (91), is activated after MPTP intoxication to mice in response to MPTP-induced oxidative DNA damage (Fig. 3) (23, 56, 70). Pharmacological inhibition or genetic ablation of p53 attenuates MPTP-induced Bax upregulation and dopaminergic neurodegeneration (23, 70, 92). However, p53 does not participate in Bax mitochondrial translocation in this model, neither by a transcription-independent mechanism nor through induction of the BH3-only proteins Puma or Noxa (70) (Fig. 3). Instead, post-translational activation of Bax after MPTP intoxication was found to rely on the c-Jun N-terminal kinase (JNK)-dependent activation of BH3-only protein Bim (70) (Fig. 3). In agreement with this, genetic ablation of either JNK or Bim was able to attenuate Bax activation and dopaminergic cell death in MPTP-treated mice (39, 70) (Tables 1 and 2).

Once activated, Bax is able to permeabilize the outer mitochondrial membrane and induce the release of cytochrome *c*. While the mechanism by which Bax induces MOMP is still a matter of debate, two distinct mechanisms have been proposed, one involving the opening of the so-called mitochondrial permeability transition pore complex (PTPC) and another dependent on the formation of Bax-derived channels directly into mitochondrial membranes (28). In the context of PD, Bax-induced MOMP seems to be independent of the PTPC, since (i) Bax-induced cytochrome *c* release in MPP^+-treated brain mitochondria is not responsive to the PTPC blocker cyclosporin A (70), and (ii) mice deficient for cyclophilin D, a critical component of the PTPC, do not exhibit reduced susceptibility to MPTP intoxication (70). Of note,

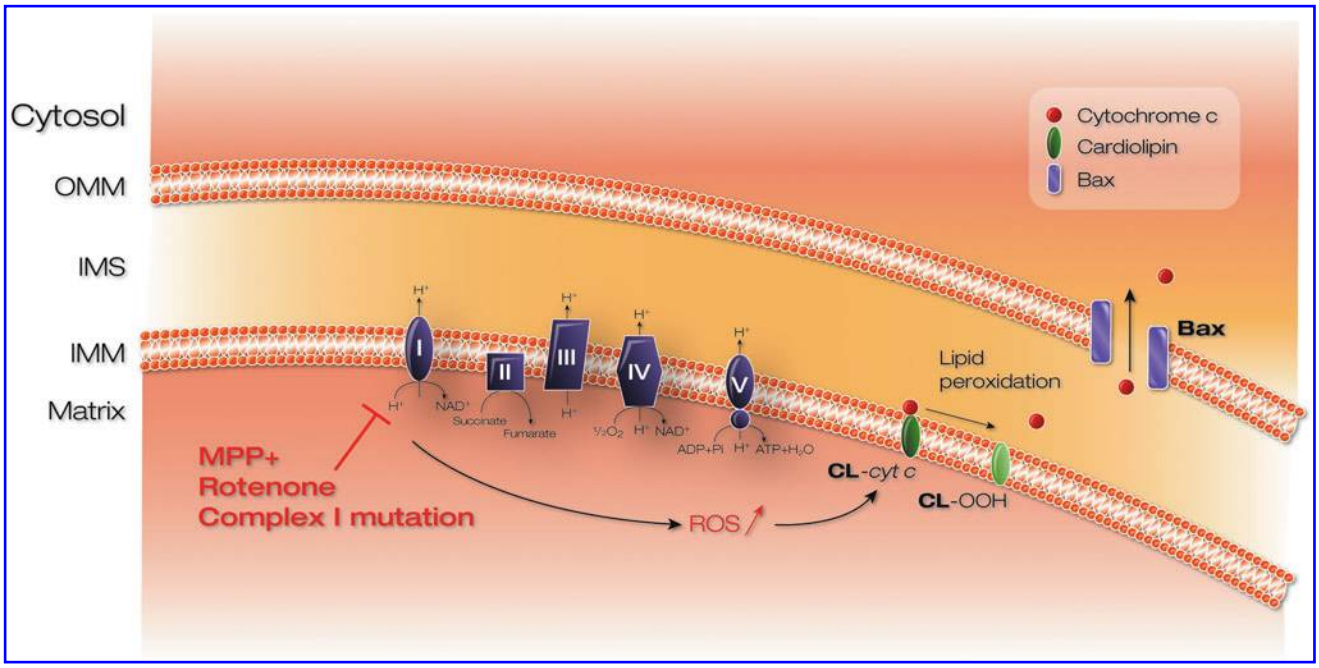

FIG. 2. Mechanism of cytochrome *c* release after complex I inhibition. Pharmacological or genetic inhibition of complex I disrupts mitochondrial respiration and stimulates the mitochondrial production of ROS. As a consequence, an array of molecules is likely oxidatively modified in response to complex I defect, including the IMM lipid cardiolipin. Cardiolipin peroxidation, in turn, affects the binding of cytochrome *c* to the mitochondrial inner membrane, leading to an increased soluble pool of cytochrome *c* in the intermembrane space. Consequently, upon permeabilization of the OMM by activated Bax, a larger amount of mitochondrial cytochrome *c* can be released, making it more likely for a compromised neuron to undergo apoptosis [adapted from Perier *et al.* (71)]. IMM, inner mitochondrial membrane; IMS, mitochondrial intermembrane space; MPP^+, 1-methyl-4-phenylpyridinium; OMM, outer mitochondrial membrane; ROS, reactive oxygen species.

while Bak is known to cooperate with Bax to promote MOMP in response to the ligation of cell- surface death receptors, it does not participate in MPTP-induced Bax-mediated dopaminergic cell death (25) (Table 1).

Based on these results, a pathogenic model of PD-related dopaminergic neurodegeneration was proposed in which neuronal death caused by complex I deficiency results from a self-amplifying cascade of deleterious events starting at the mitochondria by the alteration of the oxidative phosphorylation and finishing also at the mitochondria by the activation of the mitochondrion-dependent PCD machinery (70, 71). In this scenario (Fig. 3), MPTP-induced inhibition of complex I blocks the flow of electrons along the mitochondrial electron transport chain, resulting in increased production of ROS. Mitochondrial ROS then increase the soluble pool of cytochrome *c* in the mitochondrial intermembrane space by a mechanism involving peroxidation of cardiolipin, whereas ROS outside the mitochondria, probably also emanating from sources other than complex I inhibition (52, 53, 107), damage different cellular elements, such as lipids, proteins, and DNA. DNA damage activates both p53 and JNK. p53 induces transcriptional upregulation of Bax, whereas JNK participates in Bax mitochondrial translocation through transcriptional activation of the BH3-only protein Bim. Once localized to the mitochondrial outer membrane, Bax induces the release of cytochrome *c* into the cytosol, with ensuing caspase activation and cell death. Several elements of this molecular cascade have been demonstrated in postmortem human brain samples from PD patients, including complex I deficiency, ROS production, oxidative damage to lipids, proteins and DNA, JNK activation, Bax activation, and activation of caspase-9 and caspase-3 [reviewed in ref. (18)].

PD-Linked Genes and PCD

The potential involvement of PCD in PD has been reinforced in recent years by the finding that many of the mutated nuclear genes associated with familial forms of PD either directly or indirectly affect mitochondrion-dependent apoptosis (98) (Fig. 4).

Mutations and multiplications of α-synuclein cause autosomal dominant PD, and α-synuclein is one of the main components of Lewy bodies in sporadic PD (10, 74, 84, 85). Overexpression of α-synuclein has been shown to kill dopaminergic neurons by apoptosis *in vivo* through activation of caspase-9 and caspase-3 (109). Furthermore, despite a predominantly cytosolic and vesicular localization, a fraction of α-synuclein can also localize in mitochondria (21, 57, 68). Once in mitochondria, α-synuclein mostly associates with the inner mitochondrial membrane, where it can interact with complex

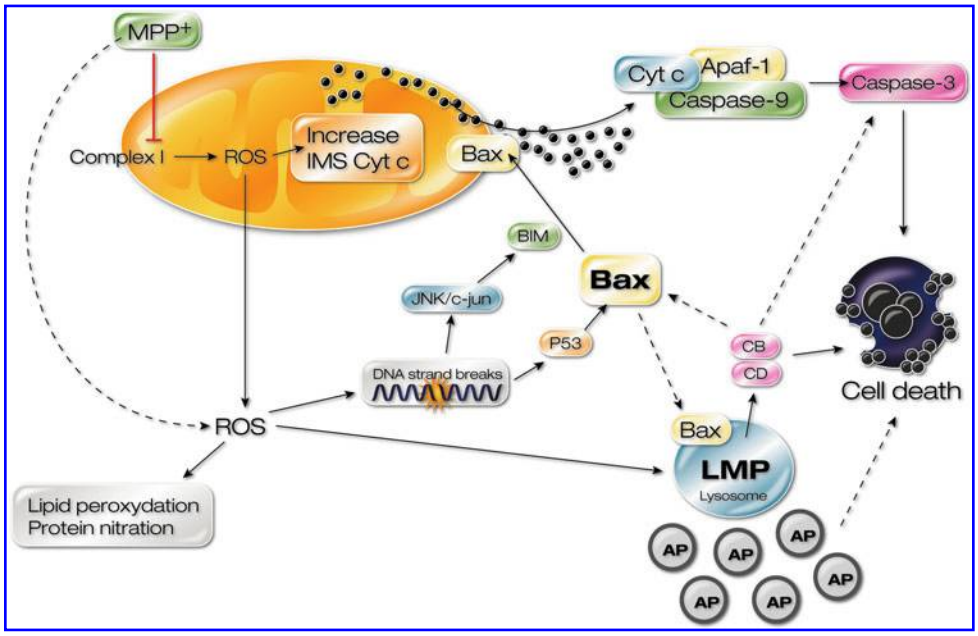

FIG. 3. Proposed pathogenic mechanisms of MPTP-induced dopaminergic PCD. MPP^+, the toxic metabolite of MPTP, impairs mitochondrial respiration in dopaminergic neurons by inhibiting complex I of the electron transport chain. Inhibition of complex I impedes the flow of electrons along the mitochondrial electron transport chain, resulting in an increased production of ROS. Both mitochondrial and cytosolic MPP^+-related ROS production damage cellular elements, including (i) the inner mitochondrial lipid cardiolipin, which disrupts the binding of cytochrome *c* to the IMM, thereby increasing the releasable soluble pool of cytochrome *c* in the IMS that can be subsequently released to the cytosol by activated Bax, and (ii) DNA, leading to the activation of the p53 and JNK/c-jun pathways, which coordinately promote the transcriptional and post-translational activation of Bax, respectively. Once activated, Bax is translocated into the mitochondria, where it induces the release of cytochrome *c* to the cytosol and the ensuing caspase activation and cell death. In addition, activated Bax has been shown in various pathological situations to be able to permeabilize lysosomal membranes, either directly or indirectly (6, 64). PD-related LMP occurs downstream mitochondrial-derived ROS production and results in (i) defective clearance and subsequent accumulation of altered mitochondria and undegraded AP, and (ii) leakage of lysosomal proteases into the cytosol, some of which, such as CB and CD, can remain active at neutral pH and cause the digestion of vital proteins or the activation of additional hydrolases, including caspases, or the recruitment of the pro-apoptotic protein Bax to the mitochondria. *Bold arrows* correspond to experimentally established mechanisms. *Dashed arrows* correspond to currently unknown mechanisms. AP, autophagosomes; CB, cathepsins B; CD, cathepsins D; JNK, c-Jun N-terminal kinase; LMP, lysosomal membrane permeabilization; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease.

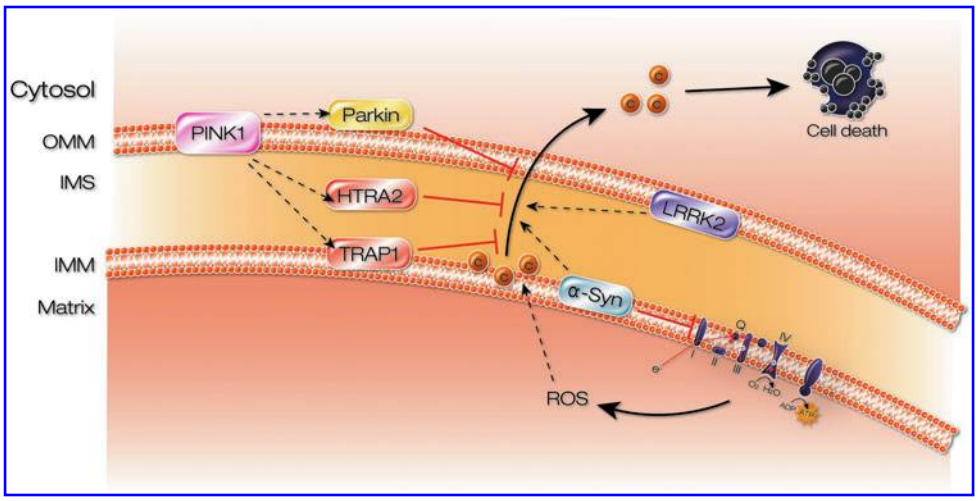

FIG. 4. Role of PD-related genes in mitochondrion-induced apoptosis. Mitochondrial PINK1 can attenuate cytochrome *c* release and apoptotic cell death by phosphorylating HTRA2 or TRAP1, two of its putative substrates. Mitochondrial parkin, in turn, can prevent mitochondrial swelling, cytochrome *c* release, and apoptotic cell death. Alpha-synuclein has been shown to directly interact with mitochondria, where it can have a deleterious role by inhibiting complex I, increasing the production of ROS, and promoting the release of cytochrome *c*. LRRK2 is partly localized to the mitochondria, where it can induce the release of cytochrome *c* and subsequent apoptotic cell death. Adapted from Vila *et al*. (98). HTRA2, high temperature requirement A2; LRRK2, leucine-rich-repeat kinase 2; PINK1, phosphatase and tensin homolog-induced kinase 1; TRAP1, tumor necrosis factor receptor-associated protein 1.

I and cause complex I deficiency and increased ROS production (21). In addition, aggregated, but not nonaggregated, α-synuclein was shown to induce cytochrome *c* release in isolated rat brain mitochondria (68). Further supporting a pro-apoptotic association between α-synuclein and mitochondria, abrogation of mitochondria DNA in yeast prevented α-synuclein-induced ROS formation and apoptotic cell death (8). A direct pro-apoptotic effect of α-synuclein may thus underlie the widespread observation that α-synuclein-deficient mice are protected against MPTP-induced dopaminergic cell death (17, 46).

Mutations in the gene encoding leucine-rich-repeat kinase 2 (LRRK2) are the most common cause of familial PD and account for up to 2% of apparently sporadic late-onset PD cases (66, 116). The subcellular distribution of LRRK2 is mostly cytosolic, but about 10% of the protein is associated with the outer mitochondrial membrane (3, 30, 103). In cell lines, PD-related mutations in LRRK2 have been shown to increase its kinase activity (103) and induce mitochondrion-dependent apoptotic cell death through the release of mitochondrial cytochrome *c* (40).

Mutations in phosphatase and tensin homolog-induced kinase 1 (PINK1) cause an autosomal recessive form of familial PD (93). PINK1 localizes into the mitochondria through an N-terminal mitochondrial targeting motif, and contains a kinase domain with the enzymatic activity to phosphorylate serine and threonine amino acid residues (83). Mutations in PINK1 are believed to impair its kinase activity and to cause PD through impaired phosphorylation of PINK1's substrates, supposedly at the level of the mitochondria. PINK1 is able to protect different cell lines against mitochondrion-dependent apoptotic cell death. Overexpression of wild-type PINK1, but not PD-associated or kinase-inactive PINK1 mutants, attenuates apoptotic cell death by reducing the mitochondrial release of cytochrome *c* and the subsequent activation of caspases (72, 102). TNFR-associated protein 1 (TRAP1) and HtrA2 were identified as the PINK1 substrates mediating the antiapoptotic effect of this protein. In cells, phosphorylation of TRAP1 by PINK1 prevented cytochrome *c* release and apoptotic cell death induced by H_2O_2 (75). The ability of PINK1 to promote TRAP1 phosphorylation and cell survival was impaired by PD-related or kinase-inactive PINK1 mutations (75). In addition, in the absence of TRAP1, wild-type PINK1 was unable to protect cells against oxidative stress-mediated apoptosis (75). Another study reported that HtrA2, a mitochondrial serine protease also known as Omi, can be phosphorylated by p38 in a PINK1-dependent manner in cultured cells (73). In addition, HtrA2 phosphorylation was shown to be virtually abolished in brains from PD patients harboring mutations in PINK1, compared with brains from sporadic PD cases (73). Phosphorylated HtrA2 protected mouse embryonic fibroblasts against 6-hydroxydopamine-, rotenone-, or stress-induced apoptosis (73). The protective effect of HtrA2 was abolished by PD-related mutations in PINK1 (73). It remains to be determined, however, whether HtrA2 and TRAP1 are authentic substrates of PINK1 *in vivo*.

Loss-of-function mutations in the gene encoding for parkin are the most frequent cause of autosomal recessive familial PD (45). Parkin has been described as an essentially cytosolic protein with E3 ubiquitin ligase activity primarily acting at the level of the ubiquitin-proteasome degradation system (97). However, in PC12 cells neuronally differentiated by nerve growth factor, parkin has been associated with the outer mitochondrial membrane, where it was shown to prevent ceramide-induced mitochondrial swelling, cytochrome *c* release, caspase activation, and apoptotic cell death (16). The anti-apoptotic effect of parkin was abolished by PD-causing parkin mutations and proteasome inhibitors, indicating that it was mediated by its E3 ubiquitin ligase activity (16).

PCD and Autophagy in PD

Autophagy is the primary mechanism by which long-lived proteins, such as α-synuclein, are degraded and is the only

mechanism by which entire organelles, such as mitochondria, are recycled. Selective autophagic degradation of mitochondria, termed mitophagy, is required for steady-state turnover of mitochondria, the adjustment of mitochondrion numbers to changing metabolic requirements or to remove damaged mitochondria (43, 112). Mitophagy consists of the sequestration of targeted mitochondria into double-membrane-bounded structures known as autophagosomes (AP), the origin of which involves the outer mitochondria membrane itself (33). Subsequently, AP fuse with lysosomes (*i.e.*, cytoplasmic membrane-enclosed organelles that contain a wide variety of hydrolytic enzymes), in which sequestered mitochondria are degraded.

Pro-apoptotic mitochondrial alterations such as loss of mitochondrial membrane potential and MOMP trigger mitophagy, in an attempt to limit the release of mitochondrial pro-apoptotic factors from damaged mitochondria (88). In PD, autophagic degradation is impaired, resulting in the accumulation of abnormal mitochondria that cannot be degraded through mitophagy (20). In such a situation, accumulation of dysfunctional mitochondrial can contribute to cell death by an increased release of mitochondrial apoptogenic factors from damaged mitochondria (63, 105). In agreement with this, pharmacological induction of autophagy with rapamycin (i) attenuated mitochondrial dysfunction, cytochrome *c* release, caspase activation, and apoptotic cell death induced by rotenone (67) or recombinant Bax (79) in cultured cells cultures, (ii) increased the survival of Drosophila intoxicated with pro-apoptotic parkinsonian neurotoxin paraquat (4, 79), and (iii) attenuated MPTP-induced dopaminergic neurodegeneration in mice (20, 54). Further supporting a role for impaired mitophagy in PD, PD-linked pathogenic mutations in PINK1 and parkin disrupt the coordinated regulatory role of these molecules at promoting autophagic degradation of dysfunctional mitochondria, thereby leading to defective mitophagy (29, 62, 101).

In sporadic PD, defective autophagy may result, at least in part (58), from a pathogenic reduction in the amount of functional lysosomes (14, 20). Decreased number of lysosomes, in turn, appears secondary to the abnormal permeabilization of lysosomal membranes induced by mitochondrially driven oxidative attack (20) (Fig. 3). The relationship between lysosomal membrane permeabilization (LMP) and apoptosis, however, has proven complex and dependent on the pathological situation (31, 47). In some cellular settings, LMP was shown to precede and contribute to the activation of mitochondrion-dependent apoptosis (6), whereas, in other situations, LMP occurred downstream MOMP and it amplified, rather than initiated, cell death (64). In addition, LMP leads to the leakage of lysosomal proteases into the cytosol, some of which, such as cathepsins B and D, can remain active at neutral pH and cause the digestion of vital proteins, the activation of additional hydrolases, including caspases, or the recruitment of the pro-apoptotic protein Bax to the mitochondria (6). Furthermore, it has been shown in various pathological settings that Bax, similar to its role on mitochondrial membranes, may also be able to permeabilize lysosomal membranes, either directly or indirectly (6, 64) (Fig. 3). These observations suggest the existence of a positive feedback loop between LMP and apoptosis that results in an overall enhancement of cell death.

Mitochondria and PCD Beyond Apoptosis

For a long time, apoptosis has been considered as the sole form of PCD, whereas necrosis was regarded as an unregulated, accidental cell death process. Morphological features of necrosis (*i.e.*, swelling of cytoplasm and organelles with moderate chromatin condensation and loss of plasma membrane integrity) have been observed in PD postmortem samples (77, 96), arguing about the potential involvement of PCD pathways in PD-related neurodegeneration. However, mounting evidence now indicates that necrosis can also occur, at least in some instances, in a highly regulated manner dependent on PCD pathways and can be prevented by anti-PCD strategies (5, 94). For instance, Bax-induced MOMP, originally considered as specifically involved in caspase-dependent apoptosis, has also been identified as a pathogenic feature of some forms of necrosis and the latter can be prevented by overexpression of Bcl-2 (5). Accordingly, apoptosis and necrosis may no longer represent two mutually exclusive cell death pathways, as they can overlap at different points (*e.g.*, mitochondria) and be mediated by similar effectors (*e.g.*, Bax). In this context, the co-existence of morphological features of both apoptosis and necrosis in PD postmortem samples is compatible with the activation of common upstream mitochondrion-dependent PCD pathways.

Apoptosis has been recurrently associated with activation of caspases. However, it is now widely recognized that PCD can also occur in the absence of caspase activation. For instance, a caspase-independent form of PCD, termed parthanatos, that depends on the activation of poly (ADP-ribose) polymerase-1 (PARP-1) has been identified (113, 114). Relevant to PD, activation of PARP-1 has been observed following MPTP intoxication (38, 55, 70) and PARP-1 ablation results in an attenuation of MPTP-induced dopaminergic cell death (55). During parthanatos, excessive PARP-1 activation leads to an intrinsic cell death program in which PAR polymer, a major product of PARP-1 activation, acts as a pro-death signal for the mitochondrion-to-nucleus translocation of AIF (113, 114). *In vitro* experimental reports have shown that AIF can be released from the mitochondria, along with cytochrome *c*, following complex I inhibition with MPP^+ in dopaminergic cell lines or primary ventral midbrain cultures (13). Furthermore, small interfering RNA-mediated knockdown of AIF in dopaminergic cell lines is able to delay MPP^+-induced cell death (13). However, whether AIF plays an actual pro-cell death role in experimental *in vivo* models of PD remains to be demonstrated, as mutant mice partially deficient for AIF not only are not protected against MPTP intoxication but are also much more sensitive to the deleterious effects of this parkinsonian neurotoxin (69). The unequivocal demonstration that AIF contributes, or not, to PD-related neurodegeneration as a pro-cell death factor will require the generation of genetically modified cell lines and mouse models in which the lethal (nuclear) functions of AIF could be dissociated from the physiological vital role of AIF in mitochondria (12, 61).

Concluding Remarks

Mounting evidence indicates that activation of mitochondrion-dependent PCD pathways plays an instrumental role in the demise of SNpc dopaminergic neurons in PD. The

complexity of this molecular cascade guarantees an exquisite control over neuronal cell death and provides several targets of potential therapeutic significance. Contrary to initial views, however, the deleterious effects of mitochondrion-mediated PCD pathways in PD may not be limited to caspase-dependent apoptosis but also extend to caspase-independent, nonapoptotic cell death, including necrosis. This notion has important therapeutic implications, as it indicates that effective neuroprotective strategies should not be limited at targeting caspase-dependent apoptosis but aimed instead at both apoptotic and nonapoptotic PCD pathways. Supporting this concept, therapeutic targeting of executioner caspases, despite initial high expectations because of the availability of pharmacological caspase inhibitors, has proven insufficient to prevent neurodegeneration as it results in the activation of alternative caspases or in a switch to caspase-independent cell death (36, 115). In contrast, targeting PCD upstream of its execution phase, such as at the level of Bax, has resulted in consistent and marked neuroprotection, even extended to axon projections (70, 71, 95). The pharmacological targeting of Bax, however, has been precluded so far by the lack of specific molecular tools with therapeutic potential.

Before considering the potential targeting of PCD pathways in PD, some considerations would need first to be addressed, such as (i) the necessity of targeting PCD pathways selectively in affected neurons but not in other type of cells; (ii) the prospective of acting at several levels of the PCD cascade to overcome the redundancy of PCD pathways; (iii) the existence of distinct self-destruction programs between cell bodies and axons (11, 78), the targeting of both of which would be required to provide symptomatic relief in PD patients; (iv) the possibility that dopaminergic neurons may be beyond rescue once the PCD cascade is already in place; or (v) the possibility that neurons may actually be rescued but no longer functional. In addition, the preclinical identification of PCD molecular targets with therapeutic potential for PD should be accompanied by the development of new clinical and pharmacological tools to allow the unambiguous assessment of the neuroprotective/disease-modifying potential of candidate drugs, which is hampered by the current inability to design clinical trials relevant to neuroprotection. For instance, it may be necessary to homogenize/stratify clinical populations before their inclusion in a given clinical trial to identify sub-groups of patients that may potentially benefit from candidate anti-PCD drugs (patients with decreased complex I activity, patients with parkin/PINK1 mutations, *etc.*). Also, in absence of early clinical markers for PD, patients may not be recruited at the earliest stages of the disease, in which a significant amount of neurons would still be preserved. Furthermore, in absence of *in vivo* indicators of neuronal dysfunction and/or death, the neuroprotective potential of candidate drugs cannot be established nor distinguished from their potential symptomatic effects. From a pharmacological point of view, the current inability to determine in advance the therapeutic doses of a candidate drug in patients, to reliably increase the blood–brain barrier crossing of specific compounds or to specifically target particular neuronal populations hinders the adequate interpretation of potentially unsuccessful neuroprotective clinical trials. Despite the challenges lying ahead, targeting of PCD pathways holds promise as a potential disease-modifying therapeutic strategy to attenuate, delay, or halt neurodegeneration in PD.

Acknowledgments

This work was supported by European Commission Marie Curie Excellence Grant (M.V.), European Commission Marie Curie International Reintegration Grant (M.V.), Fundació la Caixa, Spain (M.V.), FIS-ISCIII, Spain (M.V. and C.P.), MICINN, Spain (M.V.), and Ramón y Cajal Program from MICINN (C.P.).

References

1. Ahmadi FA, Linseman DA, Grammatopoulos TN, Jones SM, Bouchard RJ, Freed CR, *et al.* The pesticide rotenone induces caspase-3-mediated apoptosis in ventral mesencephalic dopaminergic neurons. *J Neurochem* 87: 914–921, 2003.
2. Arnoult D, Gaume B, Karbowski M, Sharpe JC, Cecconi F, and Youle RJ. Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization. *EMBO J* 22: 4385–4399, 2003.
3. Biskup S, Moore DJ, Celsi F, Higashi S, West AB, Andrabi SA, *et al.* Localization of LRRK2 to membranous and vesicular structures in mammalian brain. *Ann Neurol* 60: 557–569, 2006.
4. Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, *et al.* Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. *Cell Metab* 11: 35–46, 2010.
5. Boujrad H, Gubkina O, Robert N, Krantic S, and Susin SA. AIF-mediated programmed necrosis: a highly regulated way to die. *Cell Cycle* 6: 2612–2619, 2007.
6. Boya P and Kroemer G. Lysosomal membrane permeabilization in cell death. *Oncogene* 27: 6434–6451, 2008.
7. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, *et al.* Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest* 119: 182–192, 2009.
8. Buttner S, Bitto A, Ring J, Augsten M, Zabrocki P, Eisenberg T, *et al.* Functional mitochondria are required for alpha-synuclein toxicity in aging yeast. *J Biol Chem* 283: 7554–7560, 2008.
9. Chai J, Du C, Wu JW, Kyin S, Wang X, and Shi Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. *Nature* 406: 855–862, 2000.
10. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, lincoln S, *et al.* Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 364: 1167–1169, 2004.
11. Cheng HC, Kim SR, Oo TF, Kareva T, Yarygina O, Rzhetskaya M, *et al.* Akt suppresses retrograde degeneration of dopaminergic axons by inhibition of macroautophagy. *J Neurosci* 31: 2125–2135, 2011.
12. Cheung EC, Joza N, Steenaart NA, McClellan KA, Neuspiel M, McNamara S, *et al.* Dissociating the dual roles of apoptosis-inducing factor in maintaining mitochondrial structure and apoptosis. *EMBO J* 25: 4061–4073, 2006.
13. Chu CT, Zhu JH, Cao G, Signore A, Wang S, and Chen J. Apoptosis inducing factor mediates caspase-independent

1-methyl-4-phenylpyridinium toxicity in dopaminergic cells. *J Neurochem* 94: 1685–1695, 2005.
14. Chu Y, Dodiya H, Aebischer P, Olanow CW, and Kordower JH. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein inclusions. *Neurobiol Dis* 35: 385–398, 2009.
15. Danial NN and Korsmeyer SJ. Cell death: critical control points. *Cell* 116: 205–219, 2004.
16. Darios F, Corti O, Lucking CB, Hampe C, Muriel MP, Abbas N, *et al.* Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. *Hum Mol Genet* 12: 517–526, 2003.
17. Dauer W, Kholodilov N, Vila M, Trillat AC, Goodchild R, Larsen KE, *et al.* Resistance of alpha -synuclein null mice to the parkinsonian neurotoxin MPTP. *Proc Natl Acad Sci U S A* 99: 14524–14529, 2002.
18. Dauer W and Przedborski S. Parkinson's disease: mechanisms and models. *Neuron* 39: 889–909, 2003.
19. Degterev A and Yuan J. Expansion and evolution of cell death programmes. *Nat Rev Mol Cell Biol* 9: 378–390, 2008.
20. Dehay B, Bove J, Rodriguez-Muela N, Perier C, Recasens A, Boya P, *et al.* Pathogenic lysosomal depletion in Parkinson's disease. *J Neurosci* 30: 12535–12544, 2010.
21. Devi L, Raghavendran V, Prabhu BM, Avadhani NG, and Anandatheerthavarada HK. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem* 283: 9089–9100, 2008.
22. Dietz GP, Stockhausen KV, Dietz B, Falkenburger BH, Valbuena P, Opazo F, *et al.* Membrane-permeable Bcl-xL prevents MPTP-induced dopaminergic neuronal loss in the substantia nigra. *J Neurochem* 104: 757–765, 2008.
23. Duan W, Zhu X, Ladenheim B, Yu QS, Guo Z, Oyler J, *et al.* p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism. *Ann Neurol* 52: 597–606, 2002.
24. Eberhardt O, Coelln RV, Kugler S, Lindenau J, Rathke-Hartlieb S, Gerhardt E, *et al.* Protection by synergistic effects of adenovirus-mediated X-chromosome- linked inhibitor of apoptosis and glial cell line-derived neurotrophic factor gene transfer in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J Neurosci* 20: 9126–9134, 2000.
25. Fannjiang Y, Kim CH, Huganir RL, Zou SF, Lindsten T, Thompson CB, *et al.* BAK alters neuronal excitability and can switch from anti- to pro-death function during postnatal development. *Dev Cell* 4: 575–585, 2003.
26. Ferger B, Leng A, Mura A, Hengerer B, and Feldon J. Genetic ablation of tumor necrosis factor-alpha (TNF-alpha) and pharmacological inhibition of TNF-synthesis attenuates MPTP toxicity in mouse striatum. *J Neurochem* 89: 822–833, 2004.
27. Frezza C, Cipolat S, Martins dB, Micaroni M, Beznoussenko GV, Rudka T, *et al.* OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 126: 177–189, 2006.
28. Galluzzi L, Blomgren K, and Kroemer G. Mitochondrial membrane permeabilization in neuronal injury. *Nat Rev Neurosci* 10: 481–494, 2009.
29. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, *et al.* PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 12: 119–131, 2010.
30. Gloeckner CJ, Kinkl N, Schumacher A, Braun RJ, O'Neill E, Meitinger T, *et al.* The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Hum Mol Genet* 15: 223–232, 2006.
31. Gonzalez-Polo RA, Boya P, Pauleau AL, Jalil A, Larochette N, Souquere S, *et al.* The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. *J Cell Sci* 118(Pt 14): 3091–3102, 2005.
32. Gross A, McDonnell JM, and Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 13: 1899–1911, 1999.
33. Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK, *et al.* Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 141: 656–667, 2010.
34. Hartmann A, Hunot S, Michel PP, Muriel MP, Vyas S, Faucheux BA, *et al.* Caspase-3: a vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proc Natl Acad Sci U S A* 97: 2875–2880, 2000.
35. Hartmann A, Michel PP, Troadec JD, Mouatt-Prigent A, Faucheux BA, Ruberg M, *et al.* Is Bax a mitochondrial mediator in apoptotic death of dopaminergic neurons in Parkinson's disease? *J Neurochem* 76: 1785–1793, 2001.
36. Hartmann A, Troadec JD, Hunot S, Kikly K, Faucheux BA, Mouatt-Prigent A, *et al.* Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. *J Neurosci* 21: 2247–2255, 2001.
37. Hayley S, Crocker SJ, Smith PD, Shree T, Jackson-Lewis V, Przedborski S, *et al.* Regulation of dopaminergic loss by Fas in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J Neurosci* 24: 2045–2053, 2004.
38. Hoang T, Choi DK, Nagai M, Wu DC, Nagata T, Prou D, *et al.* Neuronal NOS and cyclooxygenase-2 contribute to DNA damage in a mouse model of Parkinson disease. *Free Radic Biol Med* 47: 1049–1056, 2009.
39. Hunot S, Vila M, Teismann P, Davis RJ, Hirsch EC, Przedborski S, *et al.* JNK-mediated induction of cyclooxygenase 2 is required for neurodegeneration in a mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A* 101: 665–670, 2004.
40. Iaccarino C, Crosio C, Vitale C, Sanna G, Carri MT, and Barone P. Apoptotic mechanisms in mutant LRRK2-mediated cell death. *Hum Mol Genet* 16: 1319–1326, 2007.
41. Irvine RA, Adachi N, Shibata DK, Cassell GD, Yu K, Karanjawala ZE, *et al.* Generation and characterization of endonuclease G null mice. *Mol Cell Biol* 25: 294–302, 2005.
42. Jones JM, Datta P, Srinivasula SM, Ji W, Gupta S, Zhang Z, *et al.* Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. *Nature* 425: 721–727, 2003.
43. Kim I, Rodriguez-Enriquez S, and Lemasters JJ. Selective degradation of mitochondria by mitophagy. *Arch Biochem Biophys* 462: 245–253, 2007.
44. Kingsbury AE, Mardsen CD, and Foster OJ. DNA fragmentation in human substantia nigra: apoptosis or perimortem effect? *Mov Disord* 13: 877–884, 1998.
45. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, *et al.* Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392: 605–608, 1998.

46. Klivenyi P, Siwek D, Gardian G, Yang L, Starkov A, Cleren C, *et al.* Mice lacking alpha-synuclein are resistant to mitochondrial toxins. *Neurobiol Dis* 21: 541–548, 2006.
47. Kroemer G and Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* 9: 1004–1010, 2008.
48. Landau AM, Luk KC, Jones ML, Siegrist-Johnstone R, Young YK, Kouassi E, *et al.* Defective Fas expression exacerbates neurotoxicity in a model of Parkinson's disease. *J Exp Med* 202: 575–581, 2005.
49. Leng A, Mura A, Feldon J, and Ferger B. Tumor necrosis factor-alpha receptor ablation in a chronic MPTP mouse model of Parkinson's disease. *Neurosci Lett* 375: 107–111, 2005.
50. Letai A, Bassik M, Walensky L, Sorcinelli M, Weiler S, and Korsmeyer S. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell* 2: 183, 2002.
51. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, *et al.* Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91: 479–489, 1997.
52. Liberatore GT, Jackson-Lewis V, Vukosavic S, Mandir AS, Vila M, McAuliffe WG, *et al.* Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. *Nat Med* 5: 1403–1409, 1999.
53. Lotharius J and O'Malley KL. The parkinsonism-inducing drug 1-methyl-4-phenylpyridinium triggers intracellular dopamine oxidation. A novel mechanism of toxicity. *J Biol Chem* 275: 38581–38588, 2000.
54. Malagelada C, Jin ZH, Jackson-Lewis V, Przedborski S, and Greene LA. Rapamycin protects against neuron death in *in vitro* and *in vivo* models of Parkinson's disease. *J Neurosci* 30: 1166–1175, 2010.
55. Mandir AS, Przedborski S, Jackson-Lewis V, Wang ZQ, Simbulan-Rosenthal M, Smulson ME, *et al.* Poly (ADP-ribose) polymerase activation mediates MPTP-induced parkinsonism. *Proc Natl Acad Sci U S A* 96: 5774–5779, 1999.
56. Mandir AS, Simbulan-Rosenthal CM, Poitras MF, Lumpkin JR, Dawson VL, Smulson ME, *et al.* A novel *in vivo* post-translational modification of p53 by PARP-1 in MPTP-induced parkinsonism. *J Neurochem* 83: 186–192, 2002.
57. Martin LJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, *et al.* Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *J Neurosci* 26: 41–50, 2006.
58. Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, *et al.* Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *J Clin Invest* 118: 777–788, 2008.
59. Mochizuki H, Goto K, Mori H, and Mizuno Y. Histochemical detection of apoptosis in Parkinson's disease. *J Neurol Sci* 137: 120–123, 1996.
60. Mochizuki H, Hayakawa H, Migita M, Shibata M, Tanaka R, Suzuki A, *et al.* An AAV-derived Apaf-1 dominant negative inhibitor prevents MPTP toxicity as antiapoptotic gene therapy for Parkinson's disease. *Proc Natl Acad SciU S A* 98: 10918–10923, 2001.
61. Modjtahedi N, Giordanetto F, Madeo F, and Kroemer G. Apoptosis-inducing factor: vital and lethal. *Trends Cell Biol* 16: 264–272, 2006.
62. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, *et al.* PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* 8: e1000298, 2010.
63. Narendra DP and Youle RJ. Targeting mitochondrial dysfunction: role for PINK1 and Parkin in mitochondrial quality control. *Antioxid Redox Signal* 14: 1929–1938, 2011.
64. Oberle C, Huai J, Reinheckel T, Tacke M, Rassner M, Ekert PG, *et al.* Lysosomal membrane permeabilization and cathepsin release is a Bax/Bak-dependent, amplifying event of apoptosis in fibroblasts and monocytes. *Cell Death Differ* 17: 1167–1178, 2010.
65. Offen D, Beart PM, Cheung NS, Pascoe CJ, Hochman A, Gorodin S, *et al.* Transgenic mice expressing human Bcl-2 in their neurons are resistant to 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. *Proc Natl Acad Sci U S A* 95: 5789–5794, 1998.
66. Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, Van Der BM, *et al.* Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* 44: 595–600, 2004.
67. Pan T, Rawal P, Wu Y, Xie W, Jankovic J, and Le W. Rapamycin protects against rotenone-induced apoptosis through autophagy induction. *Neuroscience* 164: 541–551, 2009.
68. Parihar MS, Parihar A, Fujita M, Hashimoto M, and Ghafourifar P. Mitochondrial association of alpha-synuclein causes oxidative stress. *Cell Mol Life Sci* 65: 1272–1284, 2008.
69. Perier C, Bove J, Dehay B, Jackson-Lewis V, Rabinovitch PS, Przedborski S, *et al.* Apoptosis-inducing factor deficiency sensitizes dopaminergic neurons to parkinsonian neurotoxins. *Ann Neurol* 68: 184–192, 2010.
70. Perier C, Bove J, Wu DC, Dehay B, Choi DK, Jackson-Lewis V, *et al.* Two molecular pathways initiate mitochondria-dependent dopaminergic neurodegeneration in experimental Parkinson's disease. *Proc Natl Acad Sci U S A* 104: 8161–8166, 2007.
71. Perier C, Tieu K, Guegan C, Caspersen C, Jackson-Lewis V, Carelli V, *et al.* Complex I deficiency primes Bax-dependent neuronal apoptosis through mitochondrial oxidative damage. *Proc Natl Acad Sci U S A* 102: 19126–19131, 2005.
72. Petit A, Kawarai T, Paitel E, Sanjo N, Maj M, Scheid M, *et al.* Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J Biol Chem* 280: 34025–34032, 2005.
73. Plun-Favreau H, Klupsch K, Moisoi N, Gandhi S, Kjaer S, Frith D, *et al.* The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. *Nat Cell Biol* 9: 1243–1252, 2007.
74. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, *et al.* Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276: 2045–2047, 1997.
75. Pridgeon JW, Olzmann JA, Chin LS, and Li L. PINK1 Protects against Oxidative Stress by Phosphorylating Mitochondrial Chaperone TRAP1. *PLoS Biol* 5: e172, 2007.
76. Przedborski S, Chen Q, Vila M, Giasson BI, Djaldatti R, Vukosavic S, *et al.* Oxidative post-translational modifications of alpha-synuclein in the 1- methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *J Neurochem* 76: 637–640, 2001.
77. Przedborski S, Vila M, and Jackson-Lewis V. Series introduction: neurodegeneration: what is it and where are we? *J Clin Invest* 111: 3–10, 2003.
78. Raff MC, Whitmore AV, and Finn JT. Axonal self-destruction and neurodegeneration. *Science* 296: 868–871, 2002.

79. Ravikumar B, Berger Z, Vacher C, O'Kane CJ, and Rubinsztein DC. Rapamycin pre-treatment protects against apoptosis. *Hum Mol Genet* 15: 1209–1216, 2006.
80. Rousselet E, Callebert J, Parain K, Joubert C, Hunot S, Hartmann A, *et al.* Role of TNF-alpha receptors in mice intoxicated with the Parkinsonian toxin MPTP. *Exp Neurol* 177: 183–192, 2002.
81. Saporito MS, Brown EM, Miller MS, and Carswell S. CEP-1347/KT-7515, an inhibitor of c-jun N-terminal kinase activation, attenuates the 1-methyl-4-phenyl tetrahydropyridine-mediated loss of nigrostriatal dopaminergic neurons *In vivo*. *J Pharmacol Exp Ther* 288: 421–427, 1999.
82. Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, *et al.* A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. *Dev Cell* 2: 55–67, 2002.
83. Silvestri L, Caputo V, Bellacchio E, Atorino L, Dallapiccola B, Valente EM, *et al.* Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum Mol Genet* 14: 3477–3492, 2005.
84. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, *et al.* alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 302: 841, 2003.
85. Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Roos J, and Goedert M. α-Synuclein in Lewy bodies. *Nature* 388: 839–840, 1997.
86. Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, and O'Callaghan JP. Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. *FASEB J* 16: 1474–1476, 2002.
87. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, *et al.* Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397: 441–446, 1999.
88. Tait SW and Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat Rev Mol Cell Biol* 11: 621–632, 2010.
89. Tatton NA. Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Exp Neurol* 166: 29–43, 2000.
90. Teismann P, Tieu K, Choi DK, Wu DC, Naini A, Hunot S, *et al.* Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proc Natl Acad Sci U S A* 100: 5473–5478, 2003.
91. Thornborrow EC, Patel S, Mastropietro AE, Schwartzfarb EM, and Manfredi JJ. A conserved intronic response element mediates direct p53-dependent transcriptional activation of both the human and murine bax genes. *Oncogene* 21: 990–999, 2002.
92. Trimmer PA, Smith TS, Jung AB, and Bennett JP Jr. Dopamine neurons from transgenic mice with a knockout of the p53 gene resist MPTP neurotoxicity. *Neurodegeneration* 5: 233–239, 1996.
93. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, *et al.* Hereditary Early-Onset Parkinson's Disease Caused by Mutations in PINK1. *Science* 304: 1158–1160, 2004.
94. Vandenabeele P, Galluzzi L, Vanden Berghe T, and Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat Rev Mol Cell Biol* 11: 700–714, 2010.
95. Vila M, Jackson-Lewis V, Vukosavic S, Djaldetti R, Liberatore G, Offen D, *et al.* Bax ablation prevents dopaminergic neurodegeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A* 98: 2837–2842, 2001.
96. Vila M and Przedborski S. Targeting programmed cell death in neurodegenerative diseases. *Nat Rev Neurosci* 4: 365–375, 2003.
97. Vila M and Przedborski S. Genetic clues to the pathogenesis of Parkinson's disease. *Nat Med* 10 Suppl: S58–S62, 2004.
98. Vila M, Ramonet D, and Perier C. Mitochondrial alterations in Parkinson's disease: new clues. *J Neurochem* 107: 317–328, 2008.
99. Vila M, Vukosavic S, Jackson-Lewis V, Neystat M, Jakowec M, and Przedborski S. Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. *J Neurochem* 74: 721–729, 2000.
100. Viswanath V, Wu Y, Boonplueang R, Chen S, Stevenson FF, Yantiri F, *et al.* Caspase-9 activation results in downstream caspase-8 activation and bid cleavage in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease. *J Neurosci* 21: 9519–9528, 2001.
101. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, *et al.* PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci U S A* 107: 378–383, 2010.
102. Wang HL, Chou AH, Yeh TH, Li AH, Chen YL, Kuo YL, *et al.* PINK1 mutants associated with recessive Parkinson's disease are defective in inhibiting mitochondrial release of cytochrome c. *Neurobiol Dis* 28: 216–226, 2007.
103. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, *et al.* Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci U S A* 102: 16842–16847, 2005.
104. Willis SN, Fletcher JI, Kaufmann T, van Delft MF, Chen L, Czabotar PE, *et al.* Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* 315: 856–859, 2007.
105. Wong E and Cuervo AM. Autophagy gone awry in neurodegenerative diseases. *Nat Neurosci* 13: 805–811, 2010.
106. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, *et al.* Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci* 22: 1763–1771, 2002.
107. Wu DC, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, *et al.* NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *Proc Natl Acad Sci U S A* 100: 6145–6150, 2003.
108. Xia XG, Harding T, Weller M, Bieneman A, Uney JB, and Schulz JB. Gene transfer of the JNK interacting protein-1 protects dopaminergic neurons in the MPTP model of Parkinson's disease. *Proc Natl Acad Sci U S A* 98: 10433–10438, 2001.
109. Yamada M, Iwatsubo T, Mizuno Y, and Mochizuki H. Overexpression of alpha-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-synuclein and activation of caspase-9: resemblance to pathogenetic changes in Parkinson's disease. *J Neurochem* 91: 451–461, 2004.
110. Yang L, Sugama S, Mischak RP, Kiaei M, Bizat N, Brouillet E, *et al.* A novel systemically active caspase inhibitor

attenuates the toxicities of MPTP, malonate, and 3NP *in vivo*. *Neurobiol Dis* 17: 250–259, 2004.
111. Yang LC, Matthews RT, Schulz JB, Klockgether T, Liao AW, Martinou JC, *et al.* 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyride neurotoxicity is attenuated in mice overexpressing Bcl-2. *J Neurosci* 18: 8145–8152, 1998.
112. Youle RJ and Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* 12: 9–14, 2011.
113. Yu SW, Andrabi SA, Wang H, Kim NS, Poirier GG, Dawson TM, *et al.* Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell death. *Proc Natl Acad Sci U S A* 103: 18314–18319, 2006.
114. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, *et al.* Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science* 297: 259–263, 2002.
115. Zheng TS, Hunot S, Kuida K, Momoi T, Srinivasan A, Nicholson DW, *et al.* Deficiency in caspase-9 or caspase-3 induces compensatory caspase activation. *Nat Med* 6: 1241–1247, 2000.
116. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, lincoln S, *et al.* Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 44: 601–607, 2004.

Address correspondence to:
Dr. Miquel Vila
Neurodegenerative Diseases Research Group
Vall d'Hebron Research Institute
Mediterranean Building
Lab. 102, Pg. Vall d'Hebron 119-129
08035 Barcelona
Spain

E-mail: mvila@ir.vhebron.net

Date of first submission to ARS Central, May 17, 2011; date of acceptance, May 28, 2011.

Abbreviations Used

AAV = adeno-associated virus vector
AIF = apoptosis-inducing factor
AP = autophagosomes
Bid = BH3-interacting domain
Bim = Bcl-2-interacting mediator of cell death
CB = cathepsins B
CD = cathepsins D
Cyt. c = cytochrome *c*
EndoG = endonuclease G
HtrA2 = high temperature requirement protein A2
IAP = inhibitor of apoptosis
JNK = c-Jun N-terminal kinase
KO = knockout
LMP = lysosomal membrane permeabilization
LRRK2 = leucine-rich-repeat kinase 2
MOMP = mitochondrial outer membrane permeabilization
MPP^+ = 1-methyl-4-phenylpyridinium
MPTP = 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine
PARP-1 = poly (ADP-ribose) polymerase-1
PCD = programmed cell death
PD = Parkinson's disease
PINK1 = phosphatase and tensin homologue-induced kinase 1
PTPC = permeability transition pore complex
Puma = p53-upregulated modulator of apoptosis
ROS = reactive oxygen species
SMAC/DIABLO = second mitochondrion-derived activator of caspases/direct-IAP binding protein with low PI
SNpc = substantia nigra pars compacta
tBid = truncated Bid
Tg = transgenic
TNFR = tumor necrosis factor receptor
TRAP1 = tumor necrosis factor receptor-associated protein 1
TUNEL = terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling
XIAP = X-chromosome linked inhibitor of apoptosis

This article has been cited by:

1. Anna R. Carta, Augusta Pisanu. 2012. Modulating Microglia Activity with PPAR-# Agonists: A Promising Therapy for Parkinson’s Disease?. *Neurotoxicity Research* . [CrossRef]
2. Patricia Rojas, Pedro Montes, Carolina Rojas, Norma Serrano-García, Julio César Rojas-Castañeda. 2012. Effect of a phytopharmaceutical medicine, Ginko biloba extract 761, in an animal model of Parkinson's disease: Therapeutic perspectives. *Nutrition* . [CrossRef]